IL7 increases targeted lipid nanoparticle-mediated mRNA expression in T cells in vitro and in vivo by enhancing T cell translation

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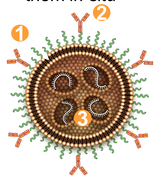
IN COLLABORATION WITH

BACKGROUND

- *Ex vivo* engineering of cells is a labor intensive and costly process
- Targeted lipid nanoparticles (tLNP) provide a useful and customizable strategy to deliver RNA to specific cell types based on the expression of cell surface markers to transfect them *in situ*



1. LIPID NANOPARTICLE
2. TARGETING ANTIBODY
3. RNA PAYLOADS

- tLNP efficacy is limited by uptake in the endolysosomes, mRNA release and / or translation of the mRNA to protein – all influenced by the metabolic and activation state of the cells
- It is unknown what 'threshold level' of transfected cells is required for therapeutic effects in both preclinical and clinical contexts.
- Therefore, we investigated whether activating or modulating T cells could improve tLNP transfection effectiveness, leading to increase protein expression levels of the mRNA cargo

METHODS

- tLNP conjugated with a CD5 targeting antibody to transfect mouse CD4+ and CD8+ T cells.
- mRNA cargo coding for the model reporter protein mCherry and detected using flow cytometry
- For RNA sequencing, CD8 T cells were isolated from the spleens of mice and cultured with IL7 or IL15 for 48 hours

1.CD5/mCherry tLNP transfect between 5-15% of T cells in vivo in naive mice

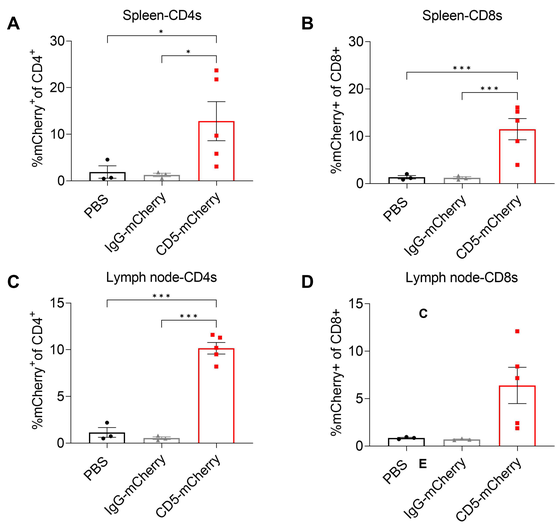


Figure 1. Mice were given 10 µg of IgG or anti-CD5-targeted LNP i.v with spleen and lymph nodes collected 24 hours later. (A-B) Percent mCherry$^+$ CD4$^+$ (A) or CD8$^+$ (B) T cells in the spleen. (C-D) Percent mCherry$^+$ CD4$^+$ (C) or CD8$^+$ (D) T cells in the lymph node. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001 '

2. Activating T cells using anti-CD3 antibodies improves tLNP-induced mCherry expression rates *in vitro* but not *in vivo*

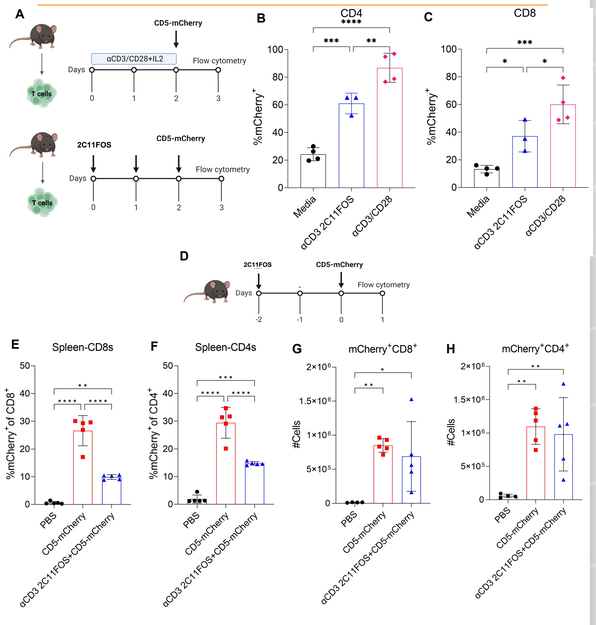


Figure 2. (A) Experimental design for in vitro T cell activation. T cells were isolated from the spleens of C57BL/6 mice, activated with either αCD3/CD28 beads + IL2 or cultured with 10 µg/ml αCD3 2C11FOS for 48 hours. Beads were removed and 1 µg of tLNP added per million cells and incubated for an additional 24 hours. (B-C) Percentage of mCherry$^+$ CD4$^+$ (B) or CD8$^+$ (C) T cells after tLNP transfection. (D) In vivo Experimental design. C57BL/6 mice were injected with 100 µg anti-CD3 2C11FOS i.p with 10 µg CD5-mCherry tLNP administered i.v 48 hours later. Flow cytometry was performed after 24 hours. (E-F) Proportion of mCherry$^+$ CD4$^+$ (E) and CD8$^+$ (F) T cells in the spleen. (G-H) Number of mCherry$^+$ CD4$^+$ (G) and CD8$^+$ (H) T cells in the spleen. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

3. IL7 enhances CD5/mCherry tLNP-induced protein expression in vitro

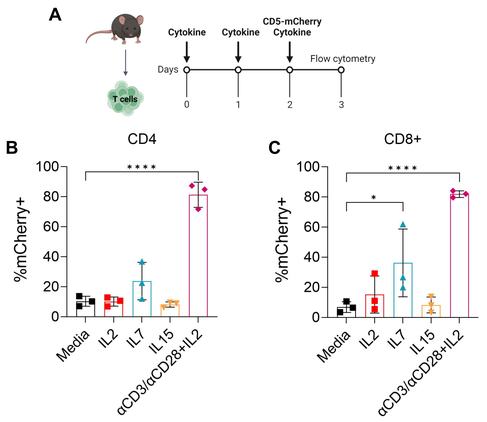


Figure 3. (A) Experimental design for in vitro cytokine treatment. T cells were isolated and cultured with either IL2, IL7 or IL15. Cytokines were refreshed daily and tLNPs added on day 2. (B-C) Proportion of mCherry$^+$ CD4$^+$ (B) and CD8$^+$ (C) T cells in vitro in the presence of IL2, IL7 or IL15. Media treated and activated T cells were used as controls. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

4. Pre-treating mice with IL7 prior to tLNPs increases both the number of transfected cells and the magnitude of protein expression

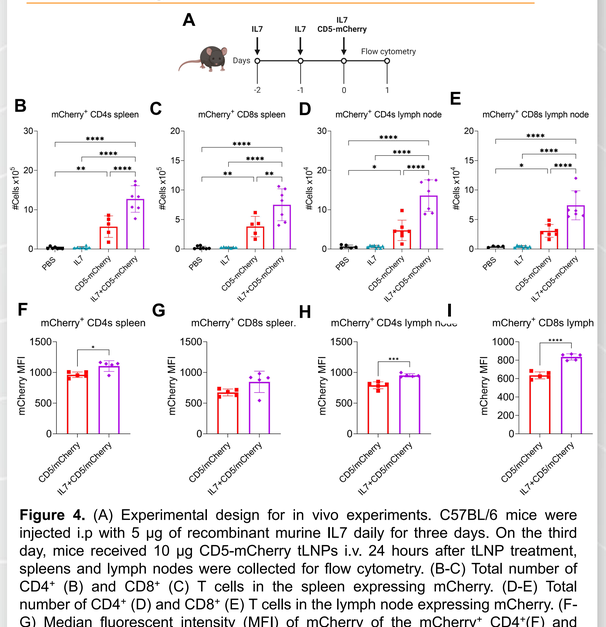


Figure 4. (A) Experimental design for in vivo experiments. C57BL/6 mice were injected i.p with 5 µg of recombinant murine IL7 daily for three days. On the third day, mice received 10 µg CD5-mCherry tLNPs i.v. 24 hours after tLNP treatment, spleens and lymph nodes were collected for flow cytometry. (B-C) Total number of CD4$^+$ (B) and CD8$^+$ (C) T cells in the spleen expressing mCherry. (D-E) Total number of CD4$^+$ (D) and CD8$^+$ (E) T cells in the lymph node expressing mCherry. (F-G) Median fluorescent intensity (MFI) of mCherry of the mCherry$^+$ CD4$^+$(F) and CD8$^+$(G) T cells in the spleen. (H-I) Median fluorescent intensity (MFI) of mCherry of the mCherry$^+$ CD4$^+$(H) and CD8$^+$(I) T cells in the spleen. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

5. IL7 increases specific changes in genes related to protein translation in CD8 T cells

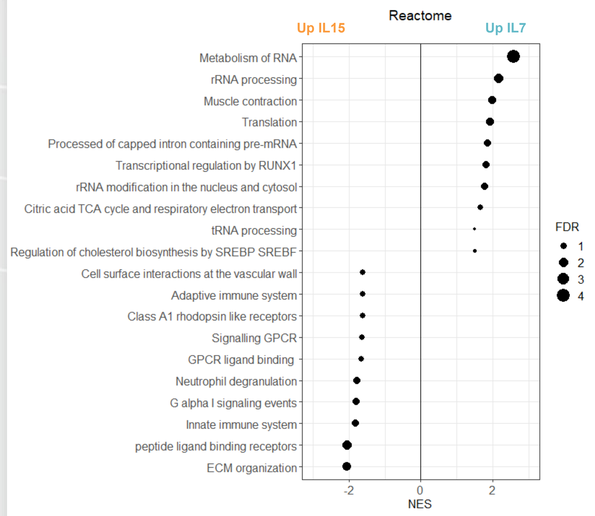


Figure 5. Gene set enrichment analysis using the list of differentially expressed genes between IL7 and IL15 treated cells using the Reactome databases. CD8 T cells were isolated from the spleens of C57BL/6 mice and cultured in either IL7 or IL15 for 48 hours. Dead cells were then removed and samples sent for RNA sequencing. Differentially expression analysis was performed using DESEQ2 with differentially expressed genes used for pathway analysis. Negative normalized enrichment score (NES) indicates pathway is upregulated in IL15 treated cells while a positive NES indicated pathway is upregulated in IL7 treated cells. FDR (-log10padj).

6. IL7 treated T cells express higher levels of mCherry after mRNA electroporation

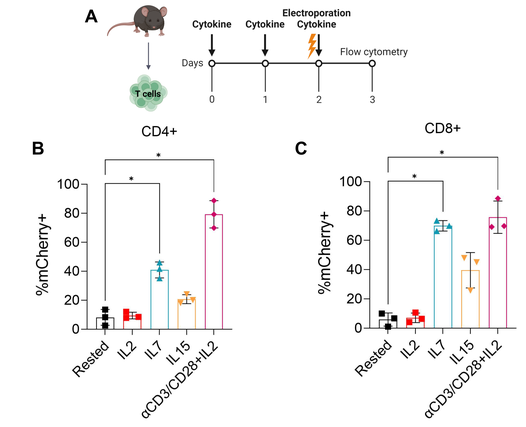


Figure 6. (A) Experimental design for electroporation experiment. T cells were isolated from the spleen C57BL/6 mice and either activated using CD3/CD28 beads or cultured in T cell media supplemented with IL2, IL7 or IL15. After 48 hours, T cells were electroporated with 2 µg of mCherry mRNA per 1 million cells. mCherry expression was measured 24 hours later. (B-C) Proportion of mCherry$^+$ CD4$^+$(B) or CD8$^+$(C) T cells after electroporation with mCherry mRNA. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

7. IL7 pre-treatment of human T cells improves CD5-mCherry tLNP-induced protein expression rates in vitro.

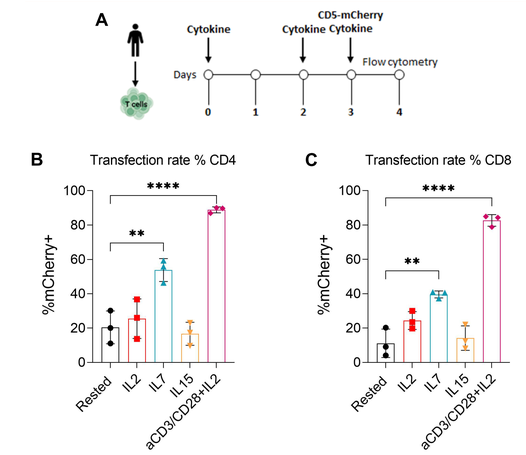


Figure 7. (A) Experimental design for tLNP transfection. Human T cells were isolated from PBMC and either activated using anti-CD3/CD28 beads with IL2 or cultured in T cell media supplemented with IL2, IL7, or IL15 and replenished after 48 hours. After 72 hours, T cells were transfected with 0.6 µg of CD5-LNP-mCherry per 2x10^5 cells. mCherry expression was measured 24 hours later. (B-C) Proportion of mCherry$^+$ CD4$^+$(B) or CD8$^+$(C) T cells after transfection with mCherry mRNA. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CONCLUSIONS

IL7 increases the protein expression of mRNA delivered by tLNPs both *in vitro* and *in vivo.*

Pathways associated with translation are upregulated in T cells treated with IL7, an observation previously unreported.